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13. ABSTRACT *(Maximum 200 words)*

The overall purpose of the research project is to develop a new method for treating metastatic prostate cancer. The new method being developed will selectively target the alpha particle-emitting radionuclide astatine-211 to metastatic cancer cells using the antibody-based approach termed "pretargeting". The goal of the first year's research effort was to optimize the reagents used, and timing for their application, in the pretargeting protocol using a prostate carcinoma xenograft model (LNCaP) in athymic mice. This goal was largely accomplished, however, some of the results obtained in the biodistribution studies suggest that minor changes in reagents will be required. Along with the synthesis of new reagents for astatine-211 labeling, six biodistribution studies were carried out. The results indicate that very good tumor targeting was obtained, but concentrations of reagents found in liver and spleen were troublesome. Therefore, we will evaluate another antibody and (possibly) another prostate cancer xenograft in continuing studies. Although some changes are anticipated, the research will continue to follow the approved "Statement of Work".

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FOREWORD

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A. Jeff Wilson 7/27/99

PI - Signature Date

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A. INTRODUCTION

This report describes the research efforts conducted during the first year of funding (July 15, 1998 thru July 14, 1999) of grant number DAMD17-98-1-8500. The research project is directed at developing a new approach to the therapy of metastatic prostate cancer. In the new approach, metastatic prostate cancer an alpha-emitting radionuclide, astatine-211, will be targeted to prostate cancer cells through an antibody-based targeting system which has been termed "pretargeting". During the first year of research the primary focus has been to develop reagents for pretargeting prostate cancer and evaluating them in a model system which is comprised of human tumor xenografts (LNCaP) on athymic (nude) mice. Significant progress has been made toward the Year 1 tasks outlined in the Statement of Work" of the proposed studies. As might be expected, some findings have suggested that additional studies should be conducted in some areas of the approach outlined in our proposal. A description of the progress made / results obtained is provided in the following section. Areas where new information indicates changes from the proposal are pointed out.

B. RESEARCH RESULTS

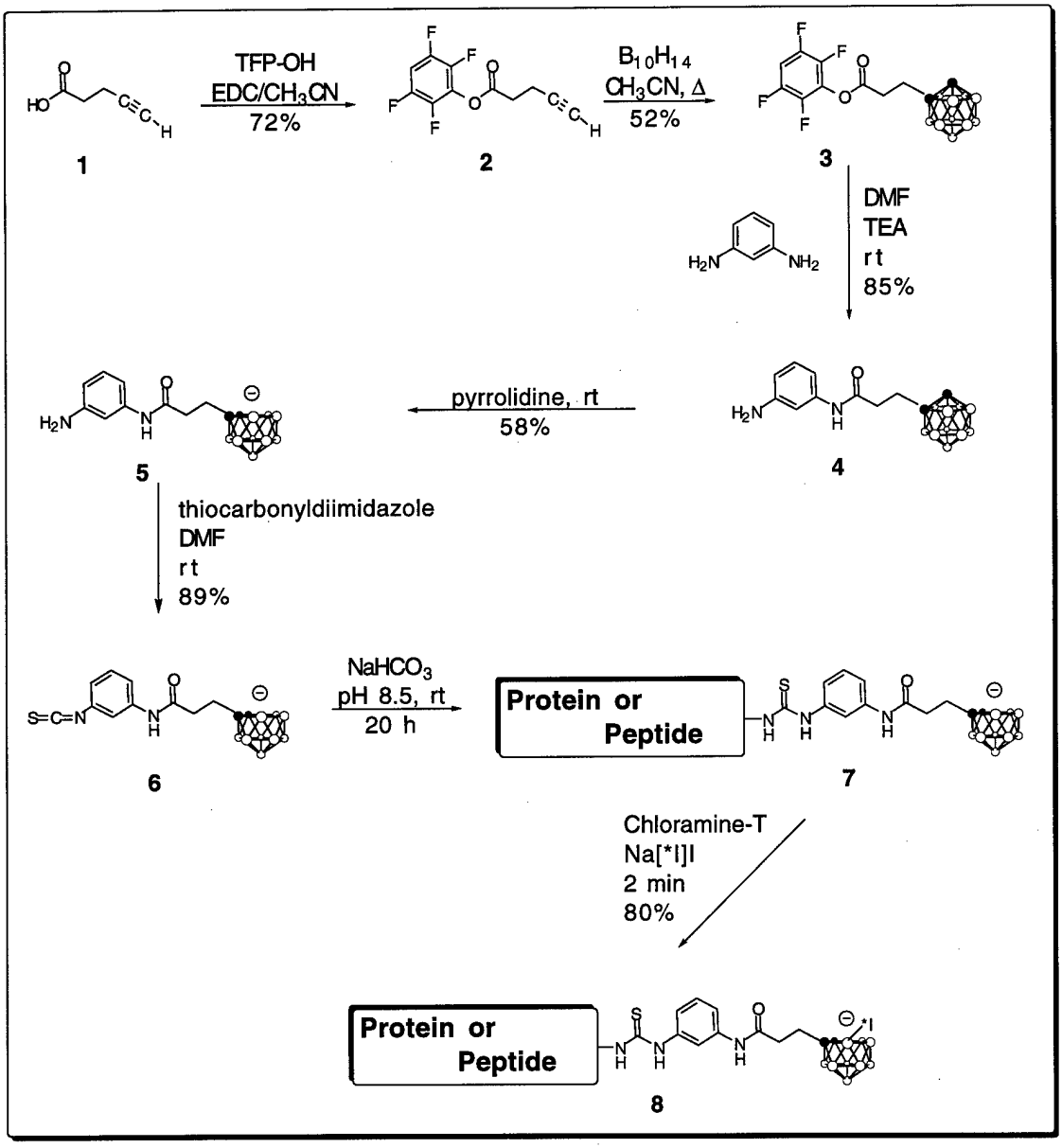
The research results are outlined below in the order of development, i.e. chemistry, in vitro testing, and in vivo testing. First the chemical steps were done, then animal studies were conducted to optimize of amounts and timing of reagent administration. The **Tasks** that each subheading addressed is included in parentheses after the subheading. **Tasks 1-6** were **completed** and **Tasks 7 and 9** were **worked on** but need additional study.

1. Development of New Reagents for Astatine Labeling (Tasks 7 and 9)

In studies prior to initiation of this research effort, we prepared several *nido*-carborane derivatives and demonstrated that they radioiodinated efficiently [1,2]. Indeed, we found that *nido*-carboranes reacted some 50x faster with radioiodine than did tyrosine. In other studies we (in collaboration with Prof. Michael Zalutsky at Duke University) demonstrated that *nido*-carboranylpropionic acid could be rapidly and efficiently astatinated [3]. Therefore, we felt that it was reasonable to consider using *nido*-carboranes that have functional groups for protein conjugation for direct introduction of radioiodine and astatine-211 into proteins. This is particularly important with astatine-211 as direct labeling results in a labile attachment of that radionuclide [4-6]. Although we attempted to prepare the *nido*-carborane conjugation reagent shown in Scheme 5 of the proposed studies, we were unsuccessful. However, during this grant period we did successfully prepare a new *nido*-carborane reagent, **6**, that can be conjugated with proteins (see Figure 1). One of the proteins that we have been interested is direct labeling of avidin. The reason for this is that we routinely have to radiolabel this protein by conjugation of radioiodinated iodobenzoate N-hydroxysuccinimide ester [7] because there are no available tyrosines to radioiodinate. The radiochemical yield for that labeling procedure is at best 60%. Thus, we have studied the conjugation of **6** with avidin and found that using more than 5

equivalents (offered) causes rapid (visible) aggregation of the protein. However, at 5 equivalents offered we obtain some *nido*-carborane conjugation and are able to obtain 80->95% radioiodination yields (get 6-29% yields of partially labile radioiodine without *nido*-carborane conjugated). This demonstrates unequivocally that direct radioiodination can be accomplished on *nido*-carborane conjugates.

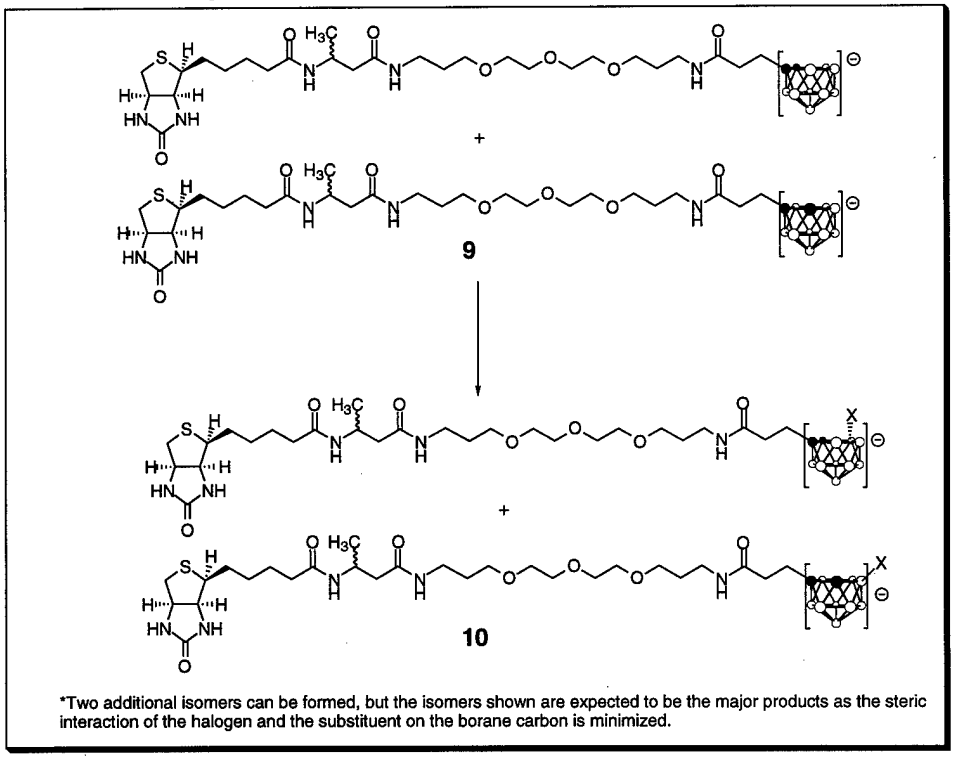
Figure 1: Synthesis, protein conjugation and radioiodination of a *nido*-carborane derivative



We have also conducted a number of conjugations of **6** with streptavidin. In contrast to the avidin reactions, streptavidin does not aggregate. We believe the propensity for avidin to

aggregate is due to its high pI (10) relative to that of streptavidin (pI = 7.2). Since there are five available tyrosines on each subunit of streptavidin (20 total) we normally directly label with radioiodine. With this number of tyrosines, it is expected that some of the radioiodine will go on tyrosines even when **6** is conjugated. Indeed, we have tried to determine what percentage goes on conjugated **6** vs tyrosine residues, but that has proven to be difficult. Our studies have shown that with increasing amounts of **6** offered, a decreasing pI value is obtained (by IEF analysis). Size exclusion HPLC analysis of avidin and streptavidin which has been conjugated with **6** show that as the amount of **6** offered increases, the shape and retention value of the peaks obtained change. This fact suggested that we might be changing the molecules quite a lot. Therefore, we also studied binding of biotin (as a biotin-cyanocobalamin adduct) with the modified proteins. Although we do not have conclusive data at this time, it appears that conjugation of **6** also significantly alters the binding of biotin. We believe we know the reason for this and have some experiments planned that might alleviate that problem. We will study directly labeling these proteins with astatine-211 during the next year.

Figure 2: Biotin derivative containing *nido*-carborane that has been synthesized and astatine-211 labeled



*Two additional isomers can be formed, but the isomers shown are expected to be the major products as the steric interaction of the halogen and the substituent on the borane carbon is minimized.

In addition to directly conjugating the astatine-binding *nido*-carborane to proteins, we have been interested in preparing biotin derivatives with this functional group attached. We previously prepared a biotin derivative **9** (as a diastereomeric pair), astatinated it, and evaluated the distribution in mice [3]. While the results were encouraging, other studies demonstrated that the biotin derivative used is susceptible to biotinidase cleavage. Therefore, we decided that it was best to develop another *nido*-carborane containing biotin reagent that was stable to serum biotinidase [8].

The biotin derivative that we chose to prepare is shown as **12** in Figure 3. In that compound there is a thiourea bond coupling the biotin and the rest of the molecule. It was thought that the thiourea would not be cleaved by the amidase-based biotinidase enzyme. The ability of a thiourea functionality to block biotinidase cleavage is presently being studied under our DOE funded grant. Most importantly, however, after preparing this compound related studies have shown that biotin moieties with a thiourea bond as in **12** loose their desired high binding affinity to avidin and streptavidin. Since **12** is synthesized we will test it, but it was felt that this compound is not optimal so an additional biotin derivative, **14** is being prepared (as in Figure 4) for study.

Figure 3: Biotinidase resistant biotin derivative containing *nido*-carborane

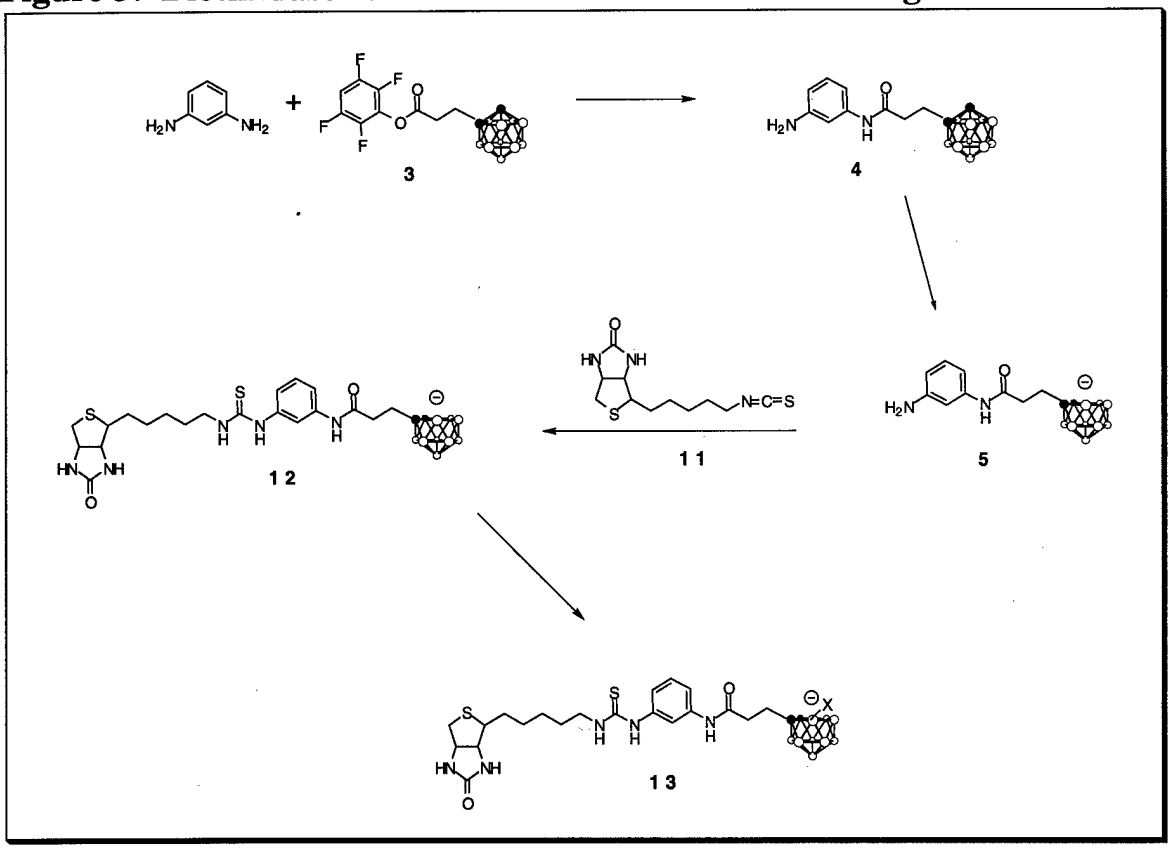
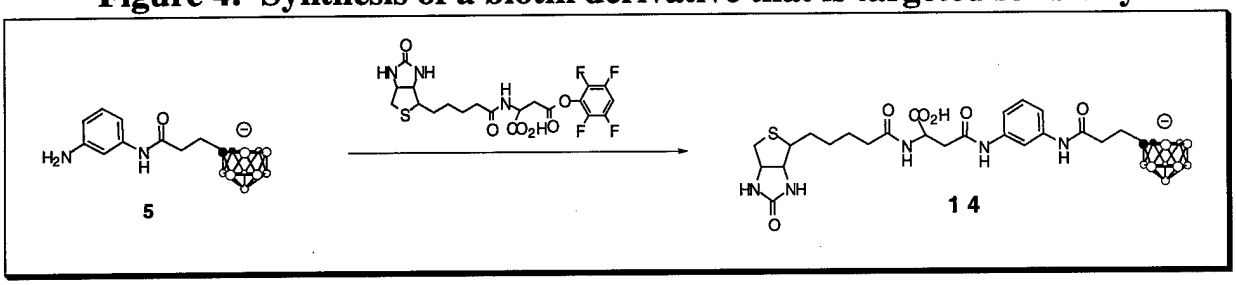


Figure 4: Synthesis of a biotin derivative that is targeted for study



2. Preparation of Reagents used in the 2-Step and 3-Step Pretargeting Procedures

A number of different compounds had to be prepared to conduct the studies proposed. Indeed, this was a large part of the "Statement of Work" in the proposal. All of the Tasks defined in the "Statement of work for Year 1 were completed except Task 7, which was to be done in the last two months of the budget year. More studies than anticipated were required in some areas which did not permit us to keep exactly to the timeline proposed. Some of the Tasks conducted are described below.

Antibody/Antigen Characterization **(Task 1)**

At the onset of this research, the prostate specific antibody, 107-1A4 developed in Dr. Vessella's laboratory had not been completely characterized. Since that time they have published an article on its characterization [9]. Even in that paper, the antigen which the monoclonal antibody 107-1A4 binds with on prostate cells had not been characterized. Very recently, it was determined that the antigen is PSMA.

Biotinylation and radioiodination 107-1A4 was the first task to be completed. Although we have developed a number of new biotinylation reagents (some with biotinidase stability), we chose to use commercially available biotin-LC NHS in these studies rather than a biotinylation reagent that we had developed because our studies indicated that there was no significant difference between them. We decided that an optimal biotin loading for the initial studies would be 2-3 biotins/mAb so that there was a minimal chance of affecting the immunoreactivity or forming aggregates. Our studies indicated that when 18 equivalents of biotin-LC NHS was added, 4.5 biotins were conjugated, and when 8 equivalents of biotin-LC NHS were used, 2.4 biotins were conjugated. A large batch conjugation of biotin-LC NHS with 107-1A4 was conducted so that the same reagent could be used in most of the animal studies conducted (except experiment where the affect of number of biotins on antibody was being evaluated).

Cell binding to Saturation Study **(Added – not defined as a Task)**

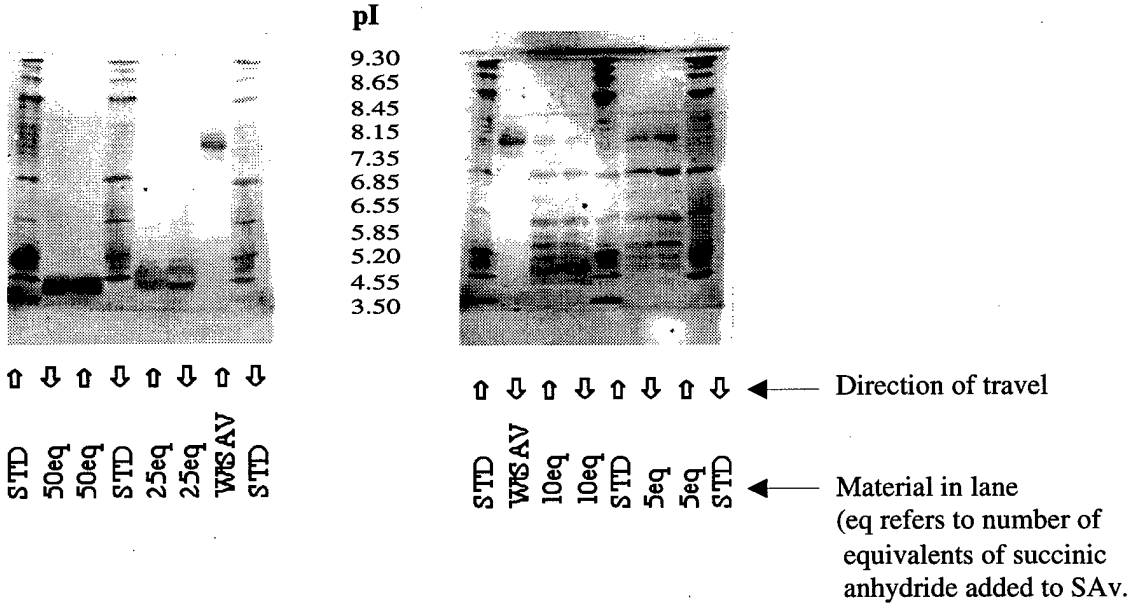
It was important to determine how many antigens are present on each cell so that we could estimate the maximum amount of antibody that we could bind to a cell. Thus, a cell saturation study was conducted with radiolabeled 107-1A4. In the experiment, increasing quantities of radioiodinated antibody were added to 10^6 LNCaP cells and the binding was assessed. A control

antibody, MOPC-21, which does not bind the cells was employed to determine non-specific binding. It was determined that there are 1-2 x 10^6 antigens per cell. This number allows sufficient loading to permit adequate numbers of astatine-211 to conduct the therapy studies.

Succinylation of Streptavidin **(Tasks 4 and 5)**

Our previous studies had demonstrated that succinylation of streptavidin decreased the kidney accumulation significantly (ref). During the studies conducted this year, a question of whether succinylated streptavidin was localizing (by itself) to the RES system arose. Since we had not previously studied how varying amounts of succinylation affected streptavidin's biodistribution, we felt that it was important to add that experiment to the proposed studies. Varying quantities of succinylation of streptavidin was obtained by reaction with 5, 10, 25 or 50 equivalents of succinic anhydride. Our previous studies had all been carried out by reaction of 50 equivalents of succinic anhydride. The IEF gels (shown below) clearly show the different levels of succinylation (and pI ranges of succinylated streptavidin) for the varying quantities of succinic anhydride offered. This material was studied in animal study 4b (see next section).

Figure 5: IEF gels of streptavidin after various levels of succinylation



3. *Animal Studies for Optimization of Pretargeting Conditions*

The first year's goals were specifically directed at Technical Objectives 1 and 2 of the proposal. Technical Objectives 1 and 2 involve optimization of the reagent quantities and time periods between reagent administrations. This optimization can only be conducted in an animal model. Our model was the LNCaP tumor xenografts in athymic mice. During the first year six animal biodistribution studies were conducted. The results of the animal studies are described below.

Animal experiment 1 **(Task 2)**

In the first animal experiment two questions were addressed. Those questions (1) How does the tumor localization of biotinylated 107-1A4 compare to that of non-biotinylated 107-1A4? and (2) How does the quantity of 107-1A4 administered affect the tumor localization? To answer the first question, equal quantities of biotinylated 107-1A4 (labeled with ^{125}I) and non-biotinylated 107-1A4 (labeled with ^{131}I) were co injected into the mice. To answer the second question, three groups of mice were injected with different total quantities of antibody (20, 50, and 100 μg). Within each group of mice, the biodistributions were evaluated at three sacrifice times. A total of 45 mice were used. The results of the experiment are shown in Figures 6a, 6b, and 6c.

It is apparent from the graphs in Figures 6a, 6b, and 6c that biotinylated 107-1A4 has a slight decrease in tumor localization (at all protein levels), but that decrease is not large enough to be a concern. Good tumor targeting and retention was obtained with all of the three quantities of 107-1A4. It was interesting to note that very similar tumor uptake was observed for even the highest quantity of antibody, suggesting that saturation had not been reached. Due to the cost of antibody in the studies it was decided from this study that a reasonable quantity to use in subsequent experiments was 50 μg per animal.

Figure 6a:
Biodistribution of 10 Micrograms each of Biotinylated [^{125}I]107-1A4 and Non-biotinylated [^{131}I]107-1A4

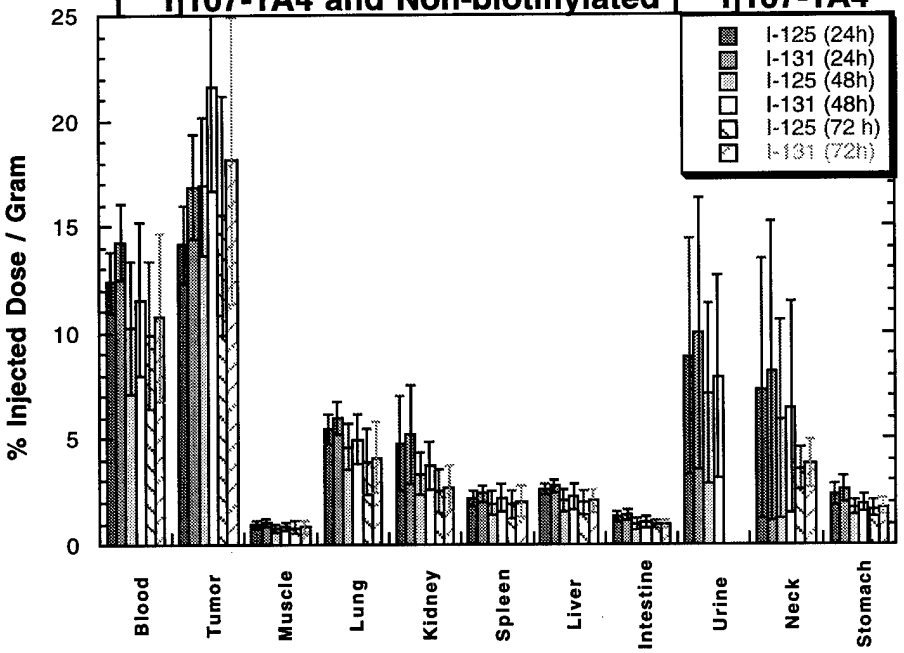


Figure 6b

Biodistribution of 25 Micrograms each of Biotinylated [^{125}I]107-1A4 and Non-biotinylated [^{131}I]107-1A4

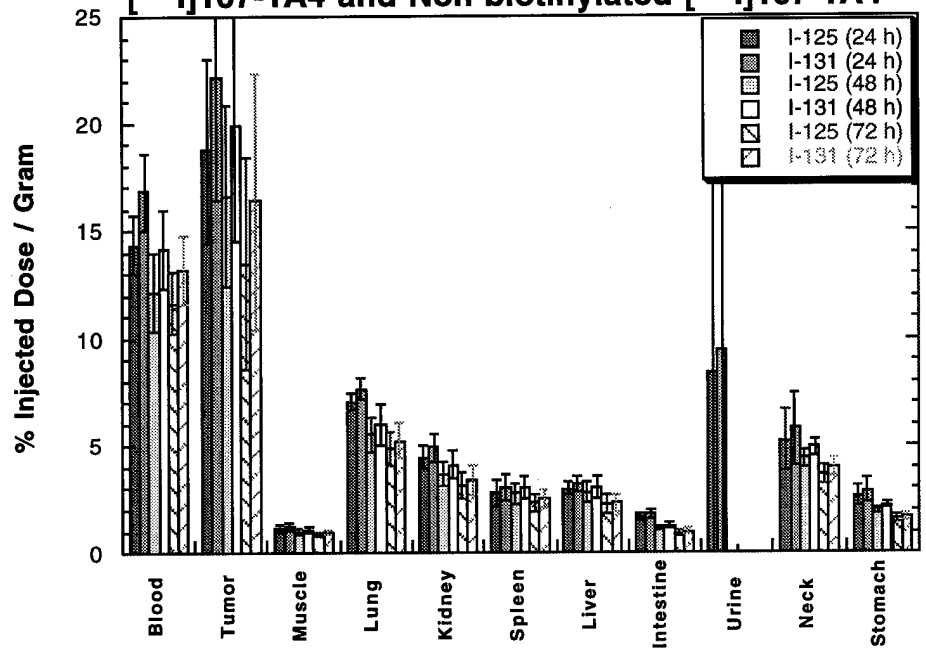
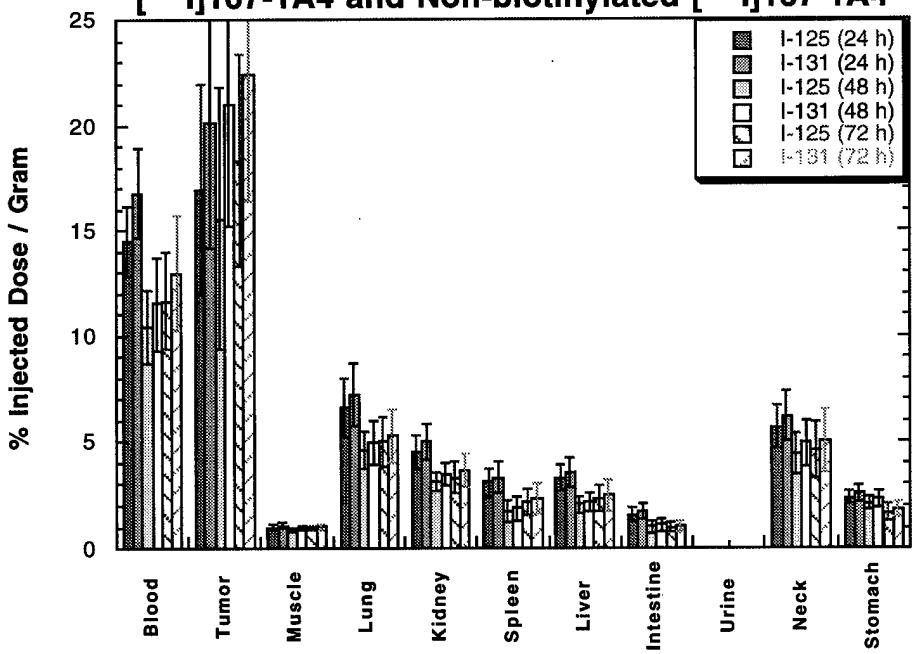


Figure 6c:

Biodistribution of 50 Micrograms each of Biotinylated [^{125}I]107-1A4 and Non-biotinylated [^{131}I]107-1A4



Animal experiment 2 **(Task 3)**

The second animal experiment was designed to optimize the amount of clearing agent, avidin, used in the two step pretargeting protocol when streptavidin is the carrier of the radionuclide. In the experiment, biotinylated [^{125}I]107-1A4 was injected and 48h later, [^{131}I]PIB-labeled avidin was injected. The blood clearance and tissue concentrations of biotinylated [^{125}I]107-1A4 were evaluated in athymic mice bearing LNCaP xenografts. Varying quantities (25, 50, 100 μg) of avidin were injected and biodistributions were evaluated at 1, 2, and 4 hours post that injection.

The results of the experiment are shown in Figures 7a, 7b, and 7c. It is clear from blood levels at 72 h post injection of the antibody that the clearing agent decreased the blood concentration 40-50% (see Figure 6b for comparison). With the exception of 4h time point, the variation at the different times after injection of avidin does not show an appreciable difference. At 4h the blood concentration appears to be higher than at the earlier times, but this might be an artifact of metabolism. As expected, the radiolabeled avidin localized in kidney, spleen, and liver. Good tumor localization of the antibody was obtained, however, the concentration at the tumor appeared to decrease with time. This might suggest that the antibody is coming off of the tumor after avidin is attached. Due to this last finding, we chose to use the smallest quantity (i.e. 25 μg) of avidin in the subsequent experiments.

Figure 7a

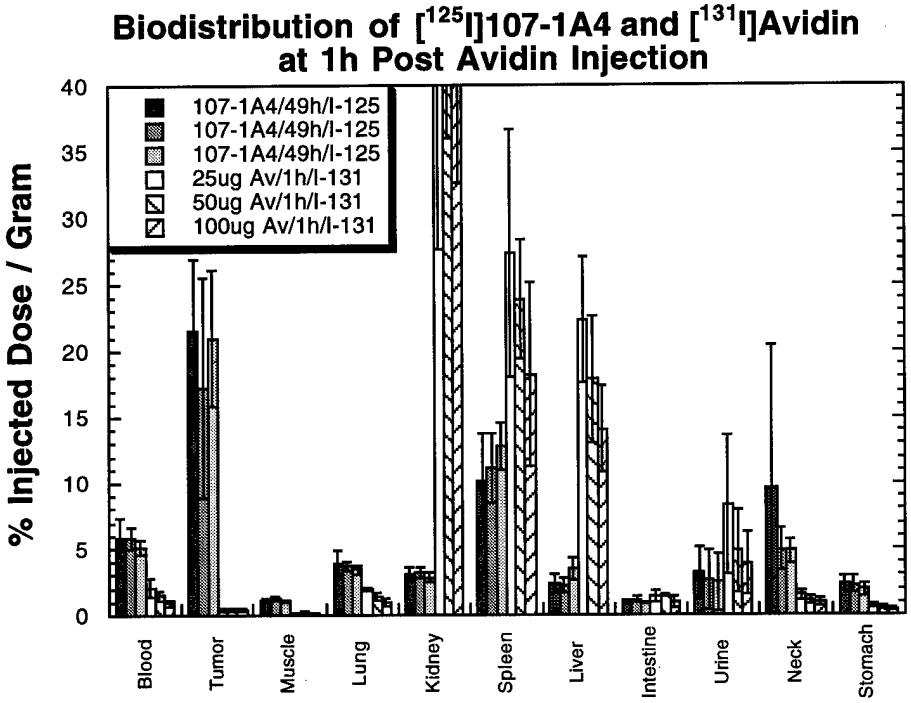


Figure 7b

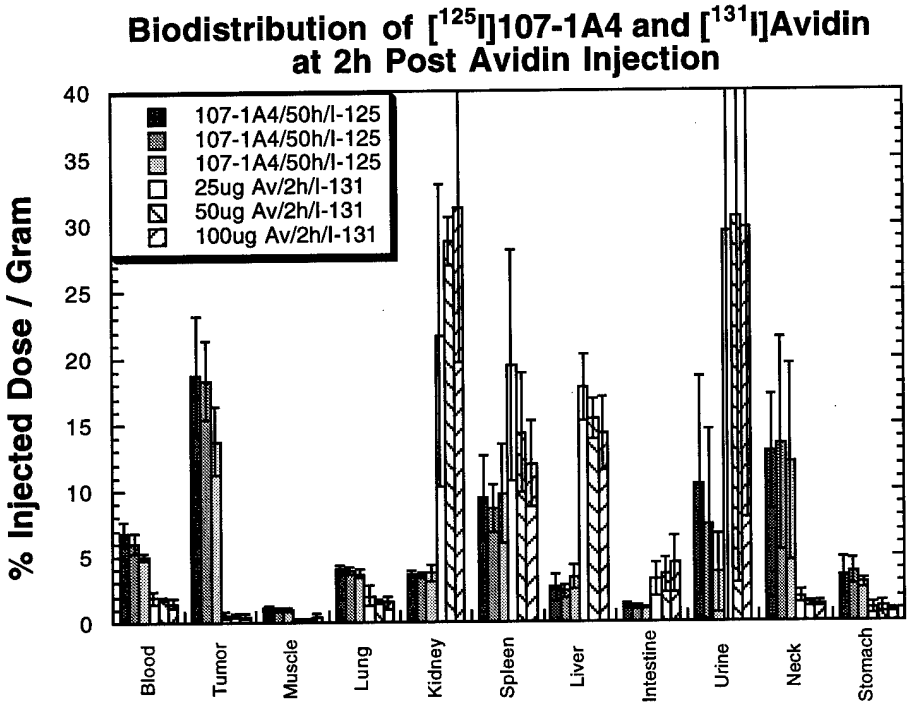
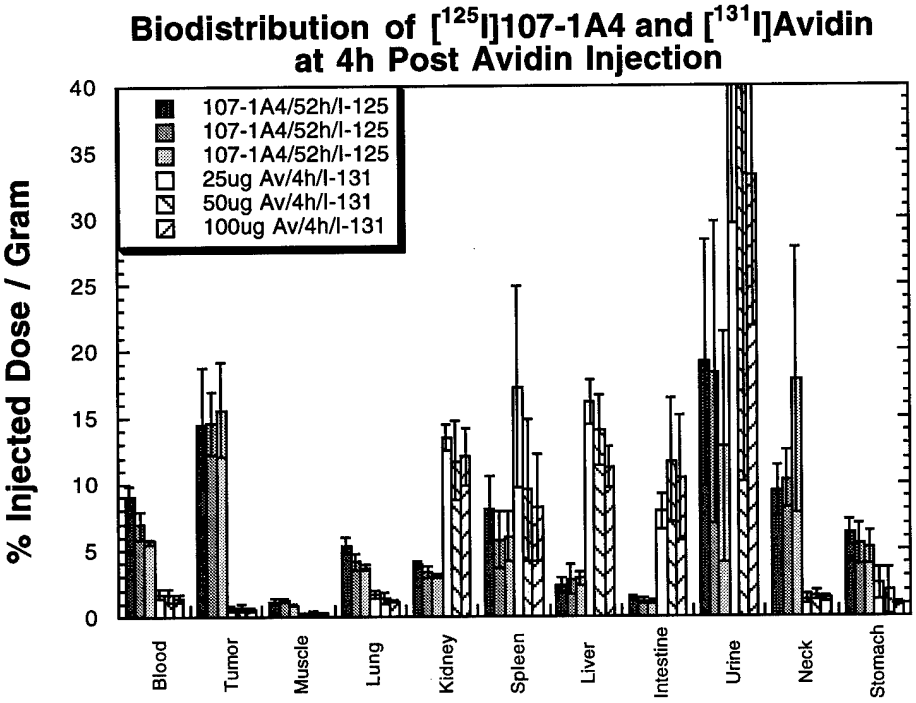


Figure 7c



Animal experiment 3 **(Task 4)**

The third animal experiment was designed to evaluate the quantity and time for injection of radiolabeled streptavidin to obtain optimal tumor co-localization of biotinylated [^{125}I]107-1A4 and succinylated [^{131}I]streptavidin in LNCaP xenografts in athymic mice when avidin is used as a clearing agent. In the experiment, varying quantities of succinylated streptavidin were evaluated at four times (1,3,7, and 24 h pi streptavidin) to determine what gave optimal tumor localization and biodistribution.

The results of the experiment are shown in Figures 8a, 8b, 8c, 8d, 8e, 8f, 8g, and 8h. Because of the large amount of data, the distribution of [^{125}I]107-1A4 and succinylated [^{131}I]streptavidin have been put on separate graphs. Thus, graphs 8a/8b, 8c/8d, and 8e/8f are pairs of the same sets of animals that have had these reagents co-injected. Graphs 8g and 8h compare the data from the experiments in a different manner to show effects. Examination of the graphs for the antibody (8a,8c,8e) shows that at time of sacrifice, the tumor-to-blood ratios of 2-3:1 were obtained. It is interesting to note that the blood levels appear to go up with time, perhaps indicating that metabolism or release of radiolabeled streptavidin is occurring. In the antibody distributions, fairly high spleen and liver concentrations are seen with all of the quantities of streptavidin used, but the quantity decreases significantly over the 24 h period.

At the initiation of this experiment, it was thought that streptavidin might localize fairly readily to the tumor xenografts, however, it is very clear from graphs 8b,8d,8f that is not the case. Indeed, not until 24 h pi streptavidin does it localize to the maximum quantity in the tumors, and at that time the tumor-to-blood ratios are similar to those obtained for the intact antibody. It is thought that this slow binding with biotinylated 107-1A4 at the tumor may be due to the highly necrotic nature of this xenograft. Another observation that can be made is that at the lower quantities of streptavidin evaluated (1 and 5 μg) the liver concentration increases with time. Thus, in our optimized studies, the minimum time after injection of radiolabeled streptavidin (for solid tumors) is 24h and the quantity used is greater than 5 μg.

To obtain a better comparison of the blood and tumor concentrations of radiolabeled streptavidin at the various times and quantities of streptavidin injected, a graph was made. That graph is shown in Figure 8g. It is very apparent from the graph that the 24 h period is best, but it suggests that there may be a problem with a short half-lived radionuclide (At-211) in solid tumor targeting with this agent. The fact that the % injected dose / gram quantity appears to decrease when the higher concentration of streptavidin was added was a bit troubling until it was assessed as pmol/g relative to the biotinylated antibody (graph 8h). Importantly, at the highest quantity of streptavidin (25 μg), a 1:1 ratio was obtained for streptavidin:antibody at the tumor.

Figure 8a

Distribution of 107-1A4, After Av, and 1,3,7,24 h Post 1 ug SAv Addition

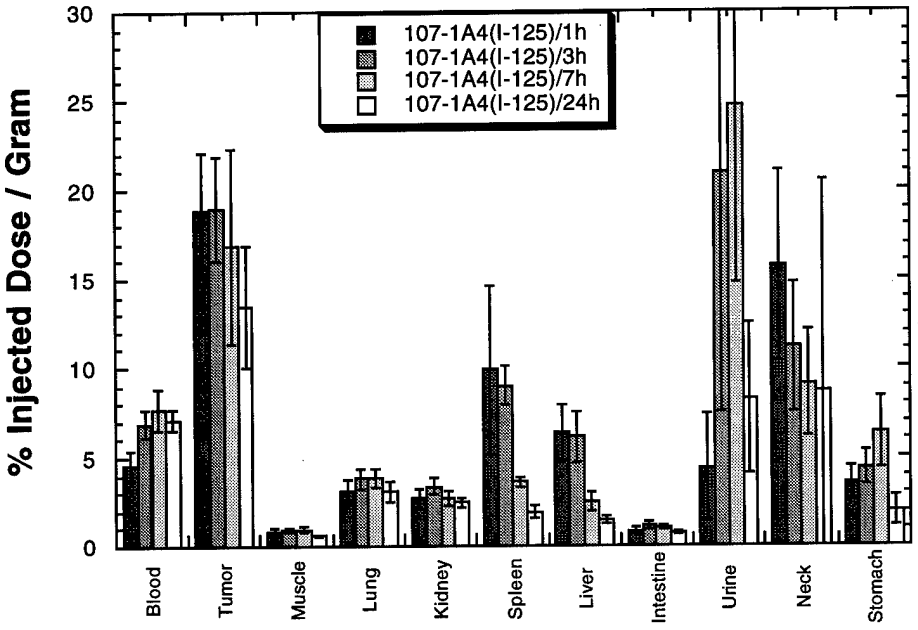


Figure 8b

Tissue Distribution of 1 Microgram SAv at 1,3,7, and 24 h post injection. (50 micrograms biotinylated 107-1A4 injected prior)

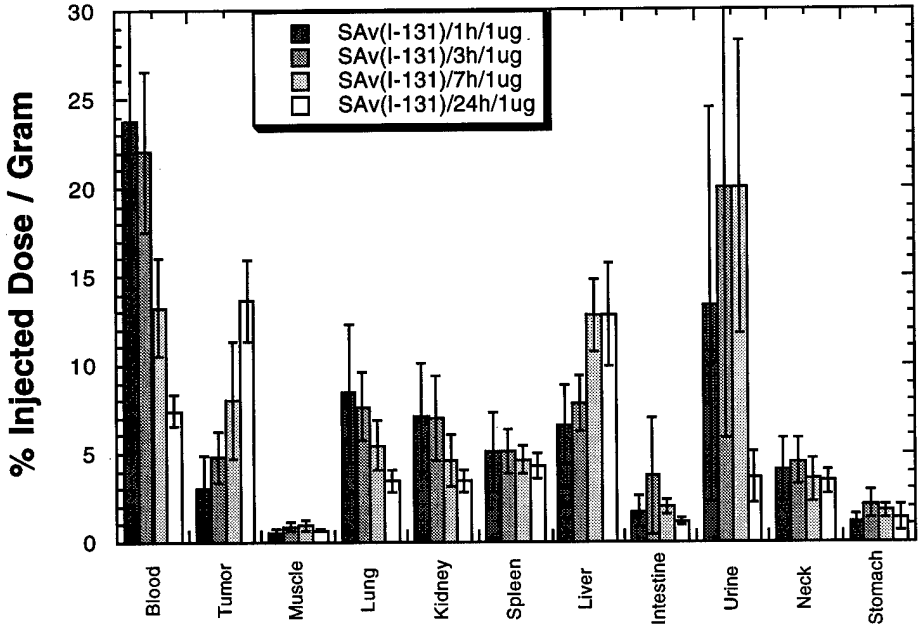


Figure 8c

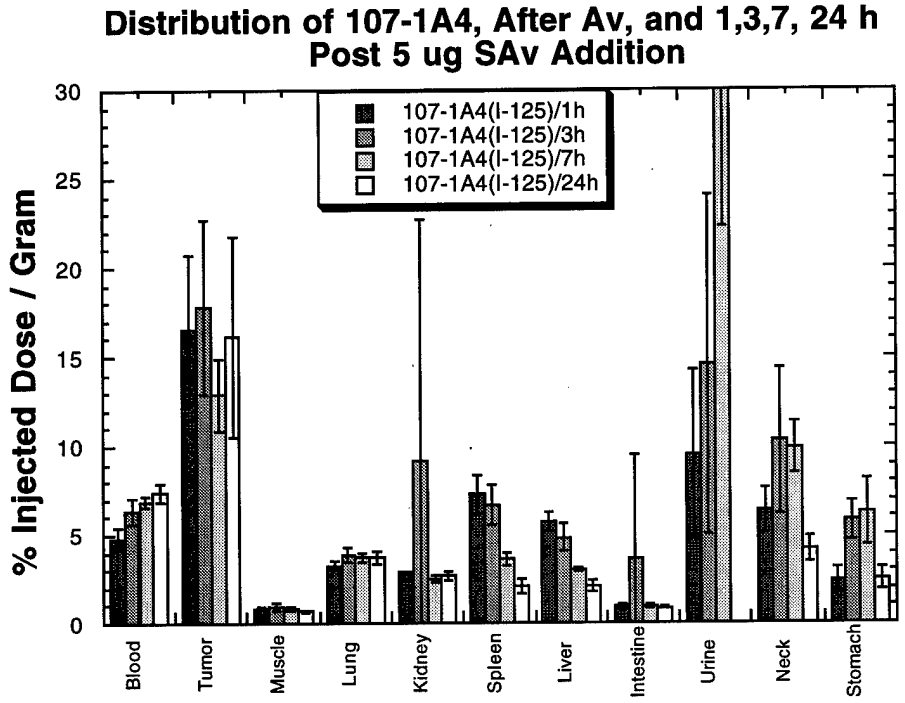


Figure 8d

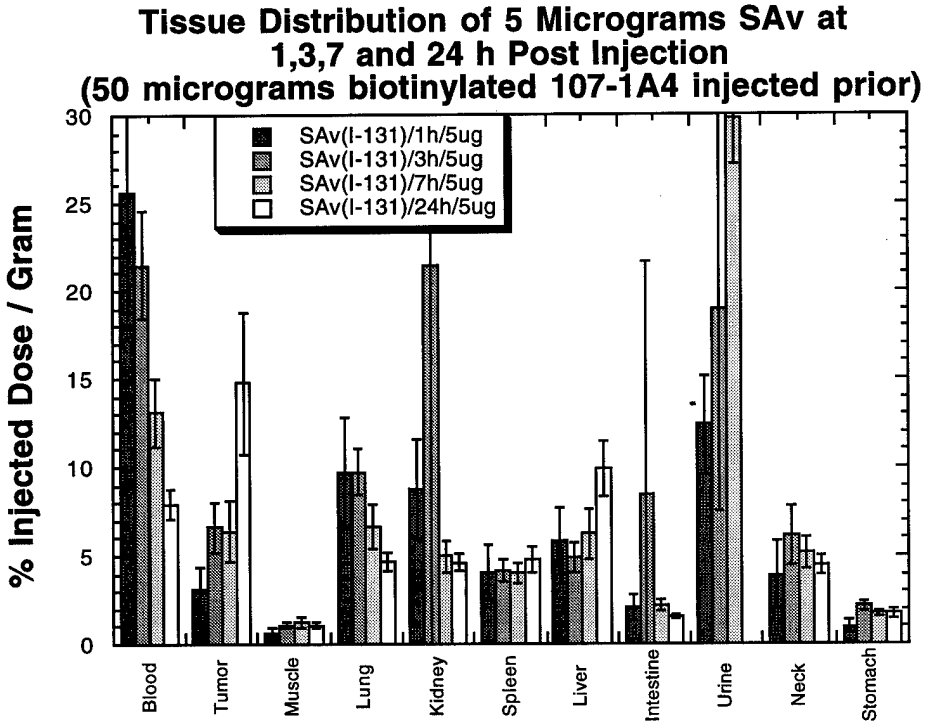


Figure 8e

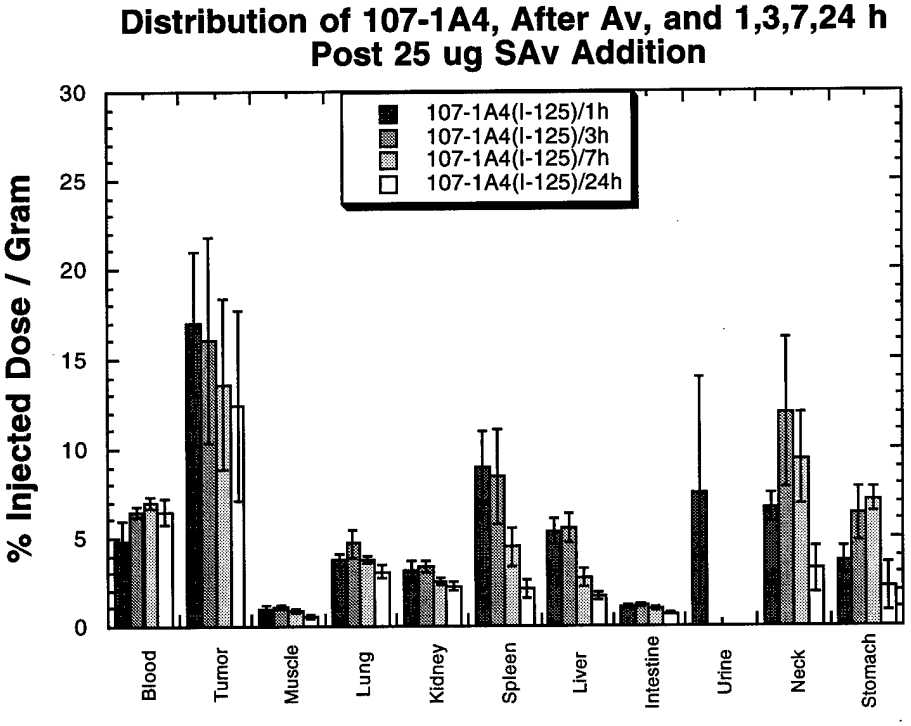


Figure 8f

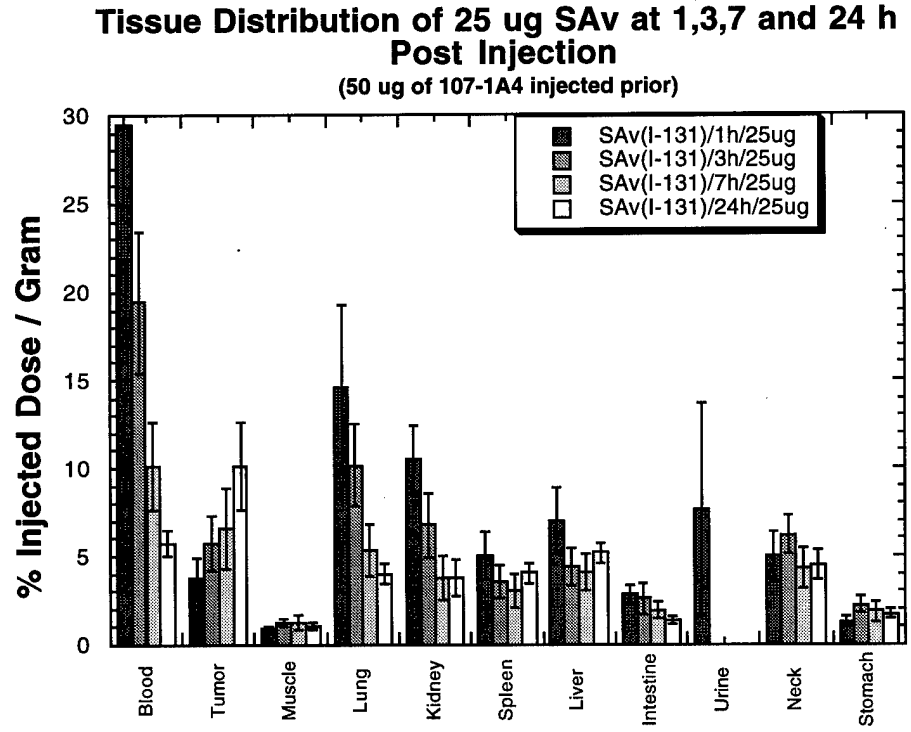


Figure 8g

Comparison of % Injected Dose / Gram of r-Streptavidin in Blood and Tumor at various times.

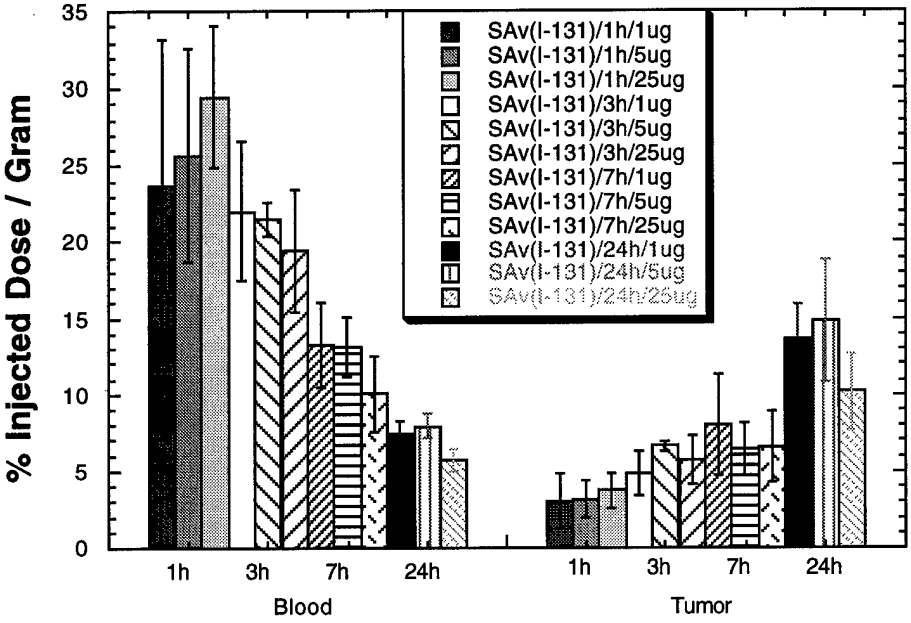
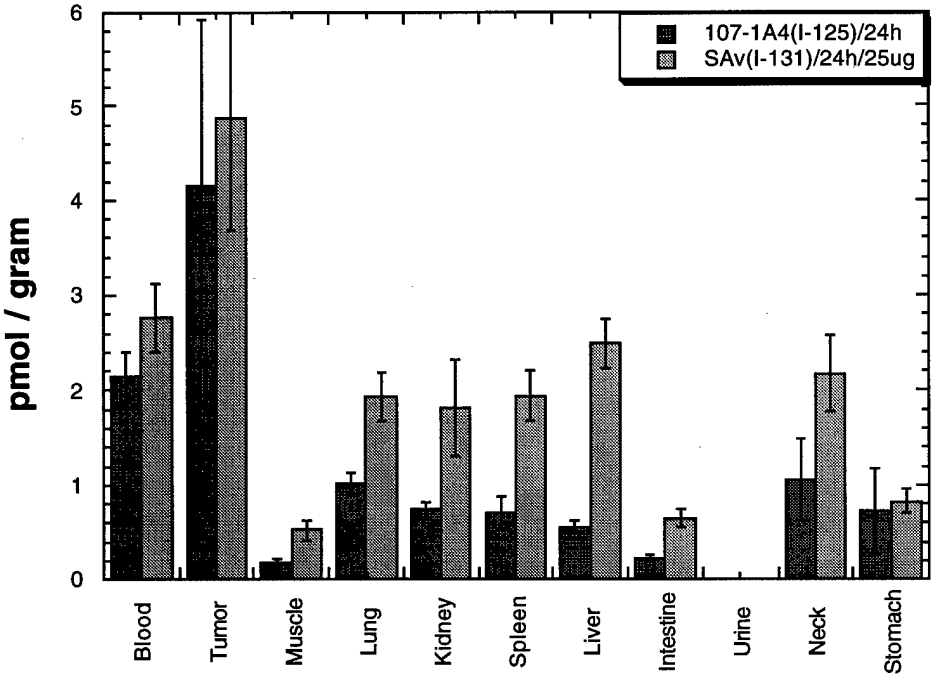


Figure 8h

Concentration (pmol/g) in Tissues of 50 ug Biotinylated 107-1A4 and 25 ug rSAv at 24h pi



Animal experiment 4 **(Task 4)**

The fourth animal study was designed to evaluate the co-localization and tumor targeting of radiolabeled streptavidin with the antibody 107-1A4 which had varying quantities of biotin conjugated. In the experiment, 50 μg of biotinylated [^{125}I]107-1A4 with 3 different conjugation levels of biotin (2.3, 4.5, and 6.8 biotins/mAb) was injected into 3 sets of 15 athymic mice bearing LNCaP xenografts (45 mice total) . After 48 h, 25 μg of avidin was injected into each mouse. After 1 h, 20 μg of [^{131}I]streptavidin was injected and animals were sacrificed at 24h, 48h, and 72 h post injection.

The results are shown in Figures 9a,9b,9c,9d,9e,and 9f. Because of the large amount of data, the distribution of biotinylated [^{125}I]107-1A4 and succinylated [^{131}I]streptavidin have been put on separate graphs. Thus, graphs 9a/9b, 9c/9d, and 9e/9f are pairs of the same sets of animals that have had these reagents co-injected. Each graph contains a comparison of localization at the various biotin conjugation levels. The different sets of graphs depict the results based on the time of sacrifice.

It is apparent from the 24 h time point (9a) that as the amount of biotin conjugated to the 107-1A4 increases, the concentration in blood and tumor decreases significantly, and the concentration in the spleen increases. With time (9c: 48h; 9e: 72h) the antibody concentration falls as would be expected, but the more rapid decrease in the blood and tumor for higher levels of biotinylation is still apparent. In contrast to this, the differences seen in blood and tumor concentrations for radiolabeled streptavidin is not as large as those seen for the biotinylated antibody. Indeed, the quantities of radioactivity are significantly higher for the streptavidin in the tumor than is measured for the biotinylated antibody. This fact may be an indicator that the antibody-streptavidin complex is internalized and the antibody is degraded much more quickly than streptavidin. It is known that streptavidin is very slowly metabolized. The other major observation about the distribution of succinylated streptavidin is that the spleen and liver are quite high, particularly in contrast to the antibody. This again may be a sign of slow degradation of the streptavidin if the antigen/antibody/streptavidin complex comes is shed and the all are broken down except for streptavidin.

The results of this experiment suggest that in this tumor xenograft, the antigen that is bound is shed and internalized. It has been shown recently that the antigen for 107-1A4 is PSMA, which is shed and internalized. Alternatively, since the amount of streptavidin in spleen and liver is much higher than the antibody, we reasoned that there may be something about the succinylated streptavidin that caused it to go to or be retained in these tissues. The animal experiment that follows this one (experiment 5) was conducted to examine the latter case.

Figure 9a

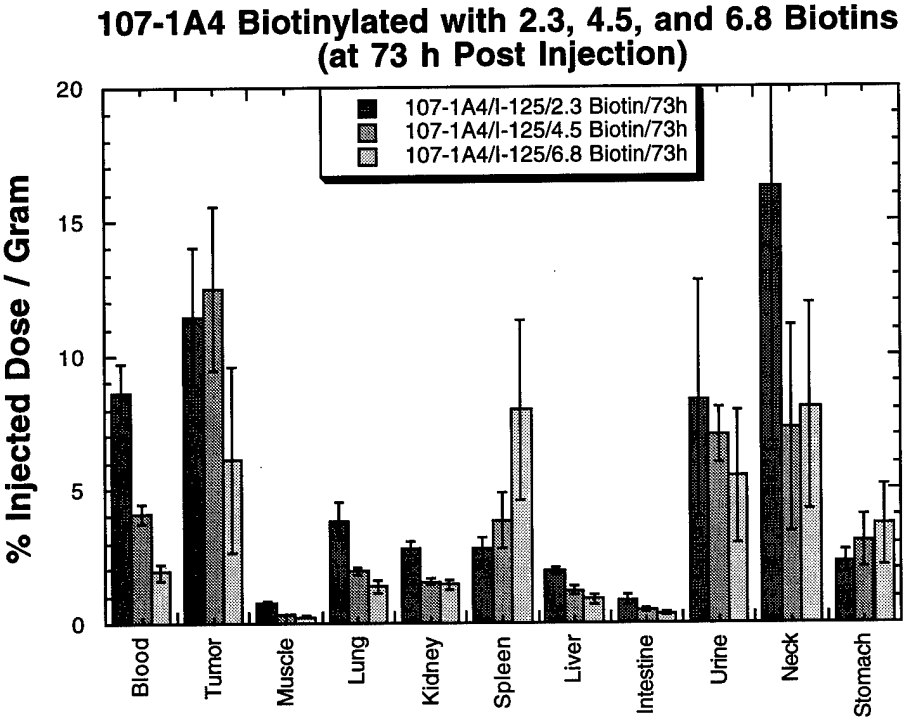


Figure 9b

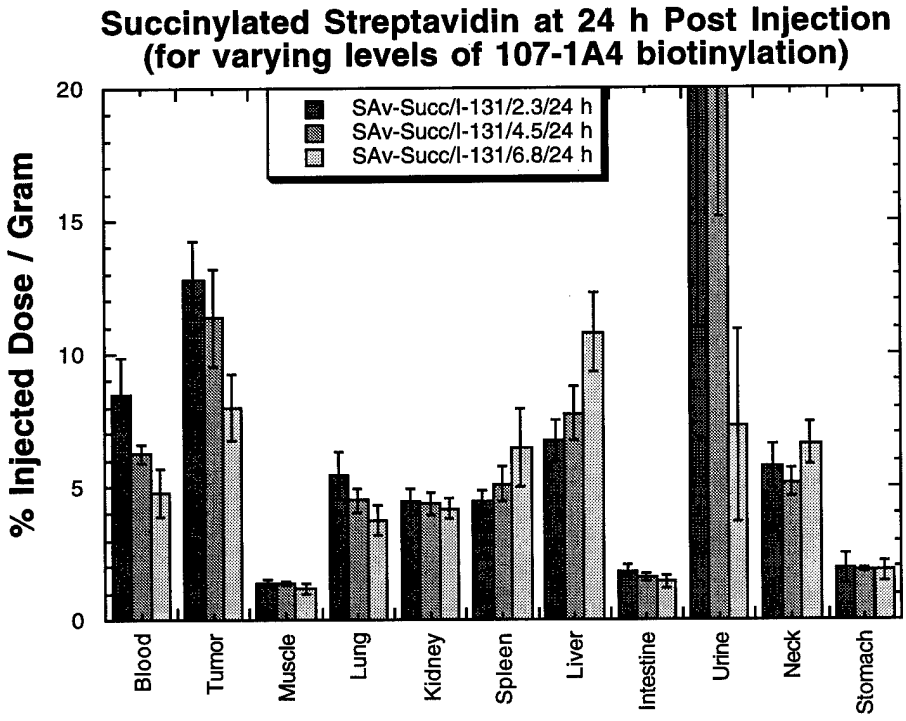


Figure 9c
107-1A4 Biotinylated with 2.3, 4.5, and 6.8 Biotins (at 97 h Post Injection)

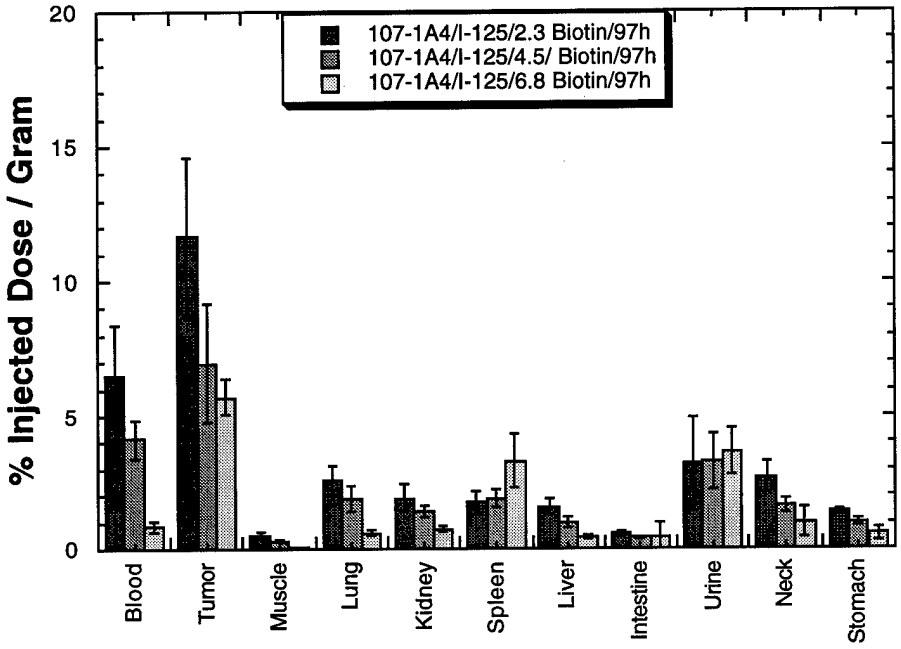


Figure 9d
Succinylated Streptavidin at 48 h Post Injection (for varying levels of 107-1A4 biotinylation)

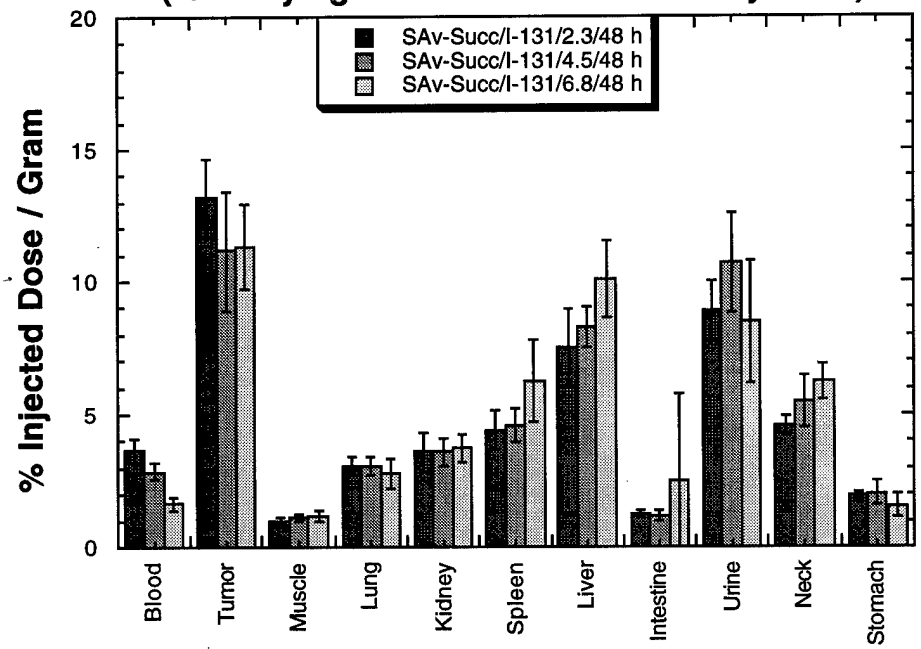


Figure 9e

107-1A4 Biotinylated with 2.3, 4.5, and 6.8 Biotins (at 121 h Post Injection)

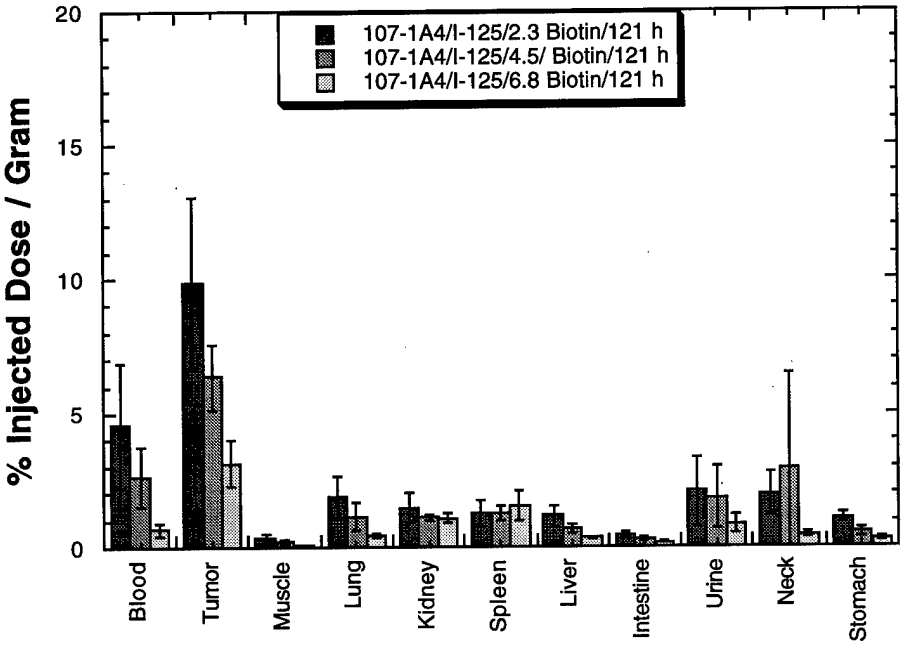
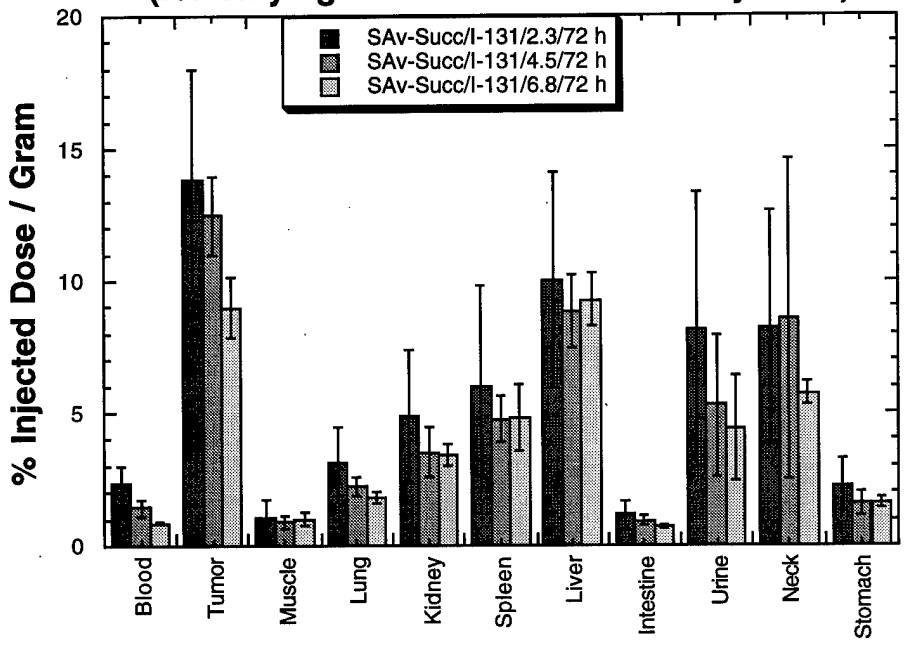


Figure 9f

Succinylated Streptavidin at 72 h Post Injection (for varying levels of 107-1A4 biotinylation)



Animal experiment 5 **(added based on results of other studies)**

The fifth animal experiment was designed to evaluate the effect of various levels of succinylation on the in vivo distribution of streptavidin. This had not been evaluated in our previous publication on succinylated streptavidin [10]. In the experiment, streptavidin which had been reacted with 5, 10, 25 or 50 equivalents of succinic anhydride were radiolabeled (4 preps) with ^{125}I. A control that contained radiolabeled [^{131}I]streptavidin was also made for co-injection. A total of 20 mice were co-injected with succinylated [^{125}]streptavidin and [^{131}I]streptavidin and biodistributions were obtained at 24 h post injection.

The results for the succinylated streptavidin by itself are shown in Figure 10a, and a comparison of the lowest and highest succinylation levels compared with unmodified streptavidin are shown in Figure 10b (unaltered streptavidin). The data indicate that the level of succinylation dramatically influences the kidney concentration of streptavidin (see Fig. 10a). The concentration of succinylated and unmodified streptavidin are very similar in all other tissues (see Fig. 10b).

Figure 10a

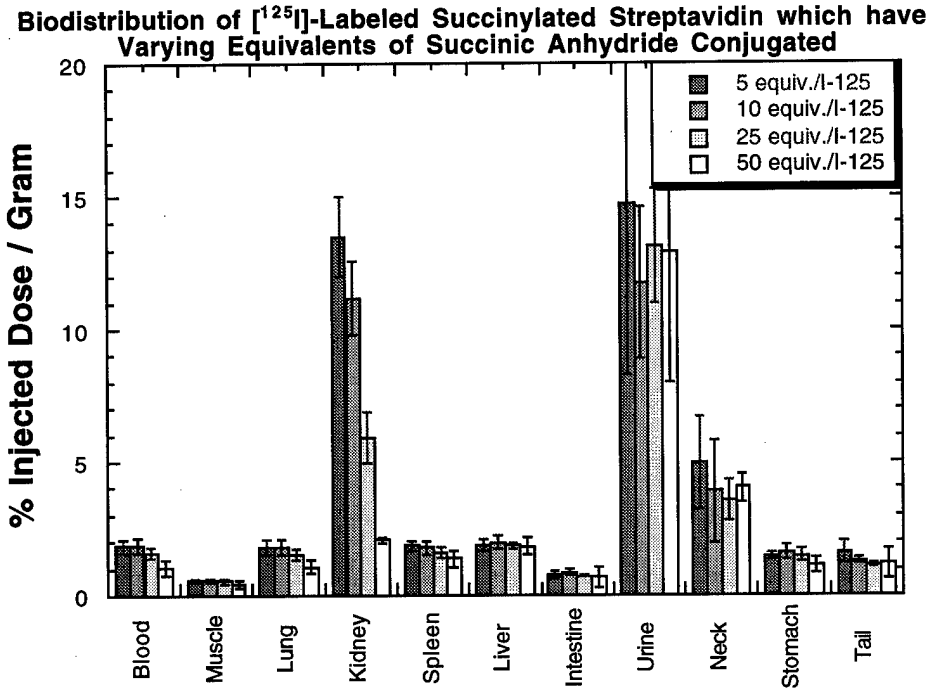
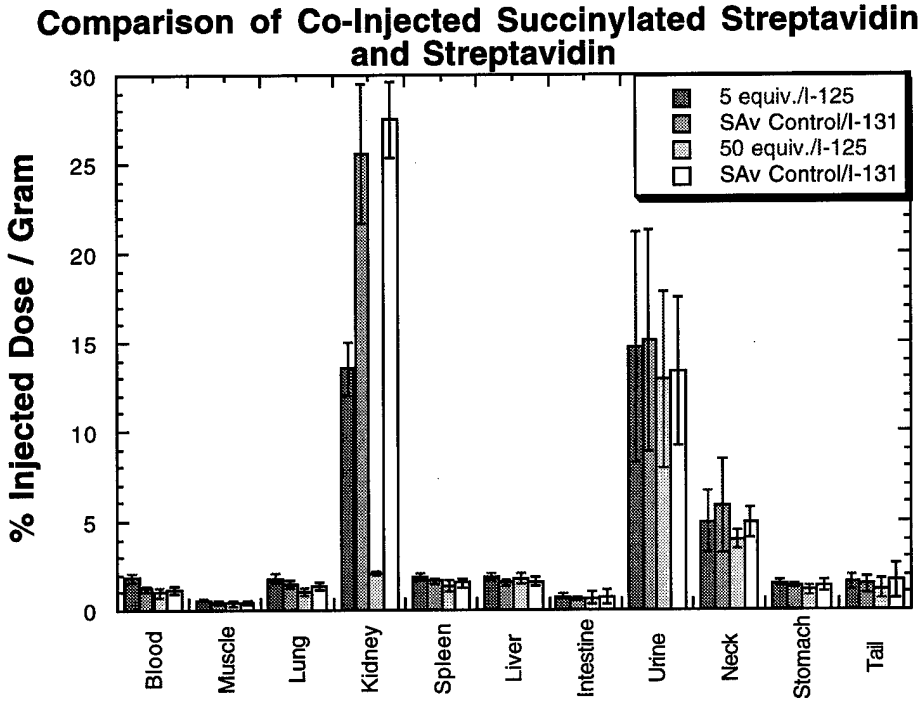


Figure 10b



The results obtained in this experiment indicate that the properties of succinylated streptavidin has little or nothing to do with the high liver and spleen concentrations observed in experiment 4. Therefore, the most logical explanation is that succinylated streptavidin was carried to those tissues as complexes with antibody or antigen/antibody complexes. We are planning to study this further by conducting the same study (or quite similar) using the renal cell carcinoma antibody, A6H, and renal cell carcinoma (RCC) xenografts, TK-82, in the near future. The antigen is not shed or internalized in the RCC xenograft.

Animal experiment 6 **(Task 6)**

The sixth animal study was designed to optimize the quantity of clearing agent used in a three step pretargeting protocol. In that study, biotinylated 50 μg of [^{125}I]107-1A4 was injected into 15 mice. After 48 h, 25 μg of [^{131}I]streptavidin was injected into each mouse. After another 24 h, varying quantities (20, 50 or 100 mg) of biotinylated asialoorosomucoid (BAOM) was injected, and 4 h later the mice were sacrificed.

The data obtained is plotted in Figures 11a ([^{125}I]107-1A4)and 11b ([^{131}I]streptavidin). The data indicate that the maximum clearance is seen even with the smallest quantity of BAOM. Plotting the concentration of reagents in picomole / gram (Figure 11c) shows that the maximum amount of streptavidin was bound (1:1 ratio) to the biotinylated antibody at the tumor.

Figure 11a

Biodistribution of 107-1A4 after 76h; 24 h after SAv and 4 h after Clearing Agent (BAOM)

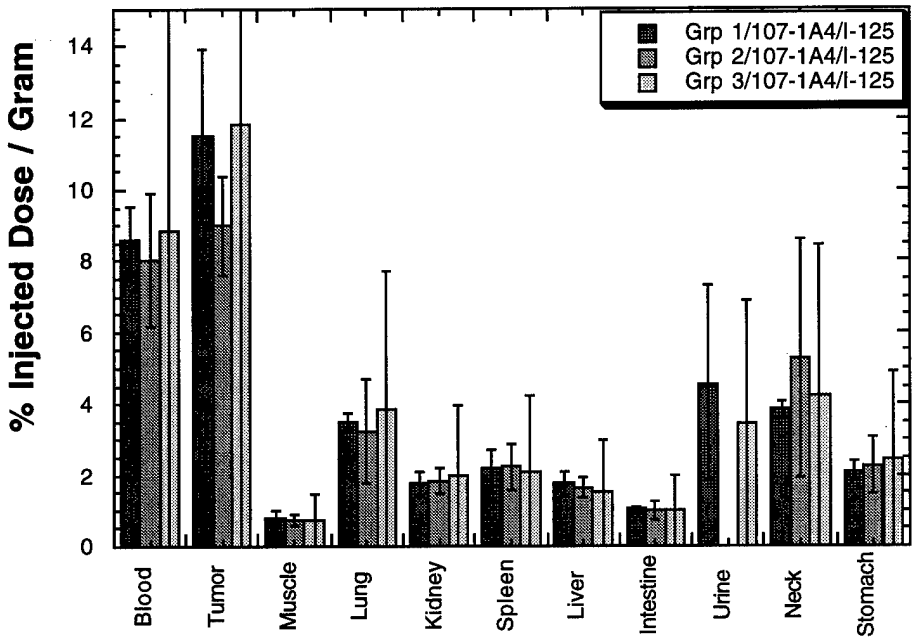


Figure 11b

Biodistribution of rSAv after 28h; 76h after biotinylated 107-1A4, and 4h after BAOM

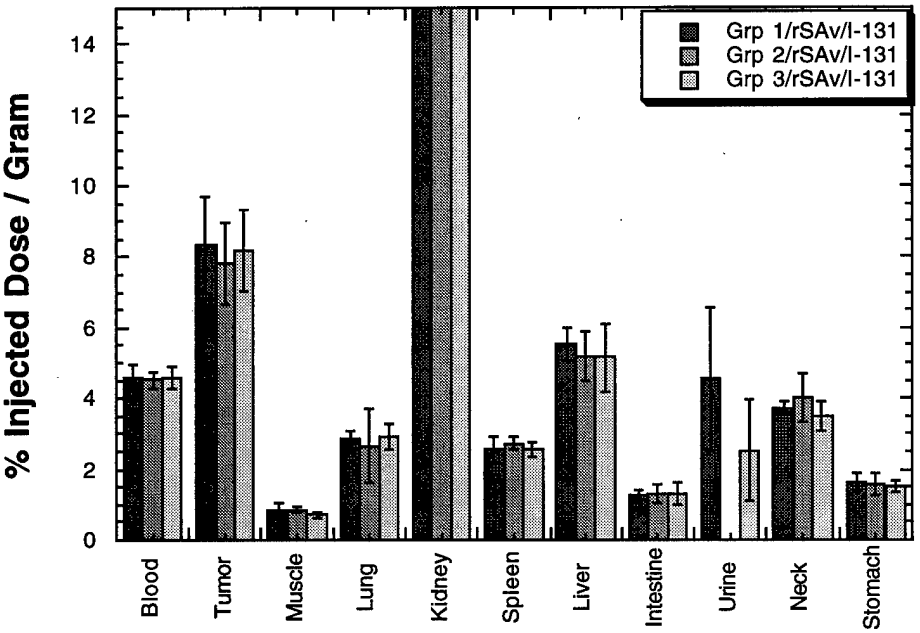
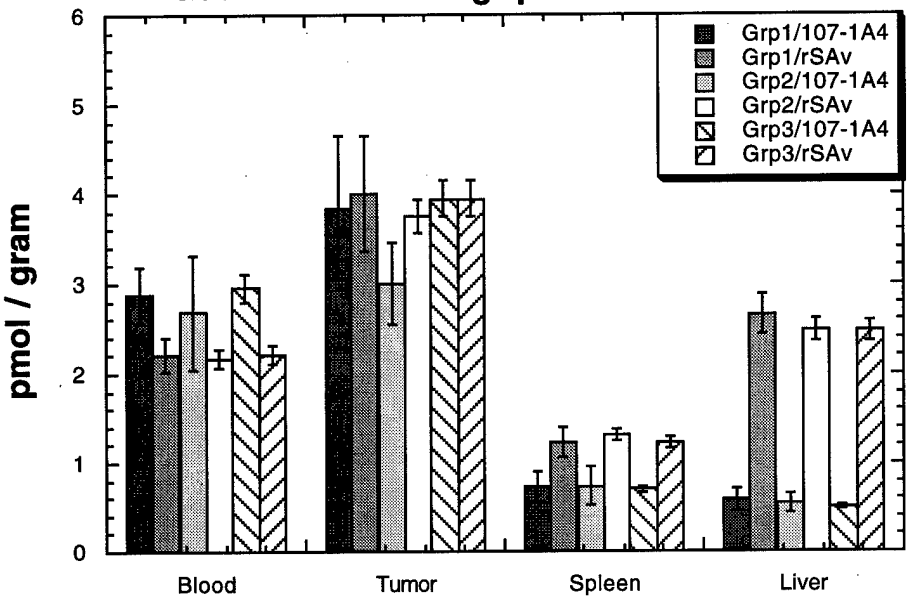


Figure 11c
Comparison of pmol of 107-1A4 and rSAv in tissues after varing quantities of BAOM



C. KEY RESEARCH ACCOMPLISHMENTS:

- Synthesized and conjugated streptavidin with the reagent, 1′-(3′-(isothiocyanato)anilino)-3-propionyl-*nido*-caborane, which is designed to permit direct labeling of proteins with astatine-211.
- Synthesized new biotin reagent which has a nido-carborane moiety for labeling with astatine-211.
- Further characterized the anti-prostate antibody, 107-1A4, and evaluated the number of binding sites per cell in the prostate carcinoma model, LNCaP.
- Successfully biotinylated 107-1A4 and evaluated its binding and in vivo characteristics at various levels of biotinylation.
- Successfully succinylated streptavidin and evaluated its in vitro and in vivo characteristics with various levels of succinylation.
- Determined the optimum amount of avidin when used as a clearing agent in the 2-step pretargeting procedure.
- Determined the optimum amount of succinylated streptavidin to obtain tumor targeting in 2-step pretargeting procedure.
- Determined the optimal amount of the biotinylated asialoorosomucoid protein required as a clearing agent in the 3-step pretargeting procedure.

D. REPORTABLE OUTCOMES:

There have been no submitted manuscripts to date on this research effort. However, it is anticipated that 2 to 3 manuscripts will be prepared and submitted for publication within the next year. The following is a list of abstracts that have been or will be presented which were funded (some in part) by this research grant.

1. **Wilbur D.S.**, Hamlin D.K., Pathare P.M., and Kegley B.B. (1999) Synthesis of a Nido-Carborane Derivative for use as a Protein Conjugate for Direct Radioiodination. Presented at the 13th International Symposium on Radiopharmaceutical Chemistry held June 27-July 1, 1999, St. Louis, MO.
2. **Wilbur D.S.**, Hamlin D.K., Pathare P.M., Chyan M.-K., and Frownfelter M.B. (1999) Optimization of Radiolabeled Biotin Derivatives for Use in Antibody Pretargeting of Cancer. Presented at the 13th International Symposium on Radiopharmaceutical Chemistry held June 27-July 1, 1999, St. Louis, MO.

G. Hamlin D.K., **Wilbur D.S.**, Pathare P.M., Venkataraman D., Corcoran M., and Press O.W. (1999) Evaluation of Antibody Biotinylation Reagents for Application to Targeted Radiotherapy. To be presented at the Third International Conference on Isotopes, to be held in Vancouver, BC, Sept. 6-10, 1999.

H. **Wilbur D.S.**, Hamlin D.K., Pathare P.M., Buhler K.R. and Vessella R.L. (1999) Evaluation of Succinylated Streptavidin as the Radionuclide Carrier in Antibody Pretargeting of Prostate Cancer. To be presented at the Third International Conference on Isotopes, to be held in Vancouver, BC, Sept. 6-10, 1999.

I. CONCLUSIONS:

The first year of studies was primarily focused on optimizing the reagents and conditions for pretargeting using the 2-step and 3-step pretargeting approaches. We had many successful experiments and we also found that some small changes in direction may be needed to accomplish the goal of comparing the 2-step and 3-step pretargeting of astatine-211 for treatment of prostate cancer metastatic to bone. Perhaps the most surprising was that the succinylated streptavidin was accumulated in spleen and liver. We had not seen this in several previous studies. However, this result appears to inherent in nature of the antigen that is targeted by 107-1A4, which is the PSMA antigen. PSMA is shed into the blood and appears to be internalized when with higher levels of streptavidin loading per biotinylated antibody (may get cross-linking). We will evaluate the biodistribution of another antibody in the LNCaP xenograft and in another prostate carcinoma xenograft (LuCaP) in the near future to determine if the high liver and spleen concentrations are obtained in those systems. If it is, then we will focus on the 3-step pretargeting approach.

F. REFERENCES:

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O. APPENDICES:

All Figures, including those of reaction schemes, electrophoresis gel scans, and biodistribution graphs have been included in the Body of this report to make it easier to follow. Thus no appendices are included.